Nicotinic Receptors in the Development and Modulation of CNS Synapses

Review

Lorna W. Role* and Darwin K. Berg†
*Department of Cell Biology and Anatomy
Center for Neurobiology and Behavior
College of Physicians and Surgeons
Columbia University
New York, New York 10032
†Department of Biology
University of California, San Diego
La Jolla, California 92093

Introduction

The molecular dissection of muscle-type nicotinic acetylcholine receptors (nAChRs) with gene cloning techniques set the stage for similar analyses of ligand-gated ion channels in brain (Numa et al., 1983). The first neuronal representative of this gene family was described in 1986 by Heinemann, Patrick, and their colleagues with the cloning of an α-type nAChR subunit gene expressed in the vertebrate nervous system (Boulter et al., 1986). In contrast with muscle nAChRs, where the primary sequences of the α and β subunits are highly conserved, the primary structures of the neuronal nAChR α and β subunits are diverse (see Le Novere and Changeux, 1995; Ortells and Lunt, 1995). The functional profiles of the resultant receptor complexes are comparably varied: neurons in both the CNS and PNS express a variety of ACh-gated channels that differ from muscle nAChRs and from one another in conductance, kinetics, ion permeability, pharmacological profile, and subcellular localization (for reviews see Sargent, 1993; Patrick et al., 1993; McGehee and Role, 1995).

Our current understanding of the expression patterns and properties of nAChRs dwarfs knowledge of the physiological role of nAChRs in the CNS. Many have pursued the hypothesis, modeled on muscle and autonomic ganglia, that nAChRs might mediate fast, excitatory transmission between CNS neurons. Documented examples in support of this idea, however, are scarce (see Clarke, 1993, and below). Despite numerous studies of nicotine addiction and nicotinic effects on behavior and cognition that clearly implicate nAChRs in CNS processing, the question remains: where and how do nicotinic nAChRs participate in higher brain functions?

This review concentrates on less traditional notions of how neuronal nAChRs may contribute to CNS physiology. Although we outline progress relating nAChR structure and subunit composition to function, our discussion focuses on the contribution of neuronal nAChRs to the development and presynaptic modulation of CNS synapses in contrast with their role as postsynaptic mediators of nicotinic transmission. For more comprehensive treatments of nAChRs, the reader is referred to several recent reviews (Bertrand et al., 1993a; Sargent, 1993; Edmonds et al., 1995; Patrick et al., 1993; McGehee and Role, 1995; Karlin and Akabas, 1996).

Neuronal Nicotinic Receptor Gene Products

Eleven genes that encode neuronal nAChR subunits have been identified in rat or chicken (for reviews see Heinemann et al., 1990a; Sargent, 1993; Patrick et al., 1993). Eight are designated as α-type (α2–α9) and three as β-type genes (β2–β4; also called non-α1–α3). Assignment to the neuronal α subunit group requires inclusion of the adjacent cysteine residues equivalent to amino acids 192 and 193 of the muscle-type α subunit (α1; see Karlin and Akabas, 1996). Homology of the neuronal β sequences with the muscle nAChR β1 is less extensive, but a subset of the neuronal β genes can substitute for β1 to produce receptors when coexpressed with the muscle α1, γ, and δ genes. Recent evidence suggests there may be additional genes encoding nicotinic receptor subunits (Pugh et al., 1995).

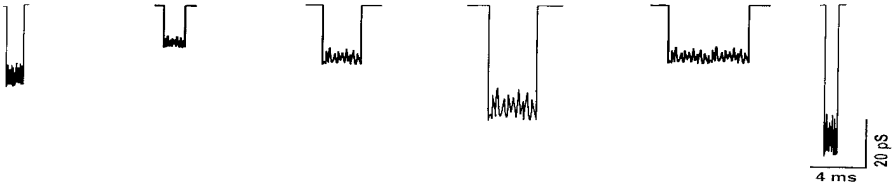
The various neuronal nAChR subunit sequences that have been identified appear to be diverse both in primary structure and in functional properties (for reviews see Sargent, 1993; Patrick et al., 1993; McGehee and Role, 1995). Heterologous expression studies reveal that many (but not all) of the α and β subunits can coassemble to form functional nAChR channels. There are clear distinctions in biophysical and pharmacological properties among the neuronal nAChR complexes studied to date, whether generated by the expression of a single α-type subunit (homomeric) or by coexpression of α and β subunits (heteromeric). Key features are summarized in Table 1 and detailed in the publications referenced therein.

Two particularly noteworthy properties of neuronal nAChR channels have emerged. First, neuronal nAChRs have a high relative permeability to Ca^{2+} compared with other ligand-gated ion channels, and second, the opening probability of neuronal nAChRs is potently modulated by external Ca^{2+} (Fieber and Adams 1991; Mulle et al., 1992a, 1992b; Sands and Barish, 1992; Vernino et al., 1992, 1994; Seguela et al., 1993; Rathouz and Berg, 1994; Castro and Albuquerque, 1995; Rogers and Dani, 1995; Amador and Dani, 1995; Dani and Mayer, 1995). Nicotinic AChRs composed of α7 subunits are more permeable to Ca^{2+} than N-methyl-D-aspartic acid (NMDA) receptor channels and insensitive to voltage-dependent Mg^{2+} blockade (e.g., Castro and Albuquerque, 1995; Rogers and Dani, 1995), thereby providing a major route of Ca^{2+} entry at rest or at hyperpolarized potentials (Mulle et al., 1992a, 1992b; Rathouz and Berg, 1994). Ca^{2+} modulation of synaptic nAChRs is discussed below.

Composition of Native Receptors

Two approaches have been used to probe the composition of native neuronal nAChRs directly. The first employs subunit-specific monoclonal antibodies (MAbs) to identify the gene products making up receptor species in vivo. The strength of this approach is that it permits analysis of minute amounts of material without requiring extensive purification. A weakness is that the functional receptor pool may comprise only a small subset of the receptors assayed. The other approach has used subunit RNA–targeted antisense oligonucleotides to test the contributions of individual subunit gene products to

Table 1. Both α- and β-Type Subunits Contribute to the Functional Diversity of Heterologously Expressed Neuronal nAChRs

	AChR Profile					
Associated Properties	α2β2	α3β2	α4β2[a]	α4β2α5[a]	α3β4	α7[a]
						
P_{Ca}/P_{Na}	~1.5	~1.5	~1.5	ND	1.1	>10
EC_{50}ACh (μM)	100	30	1	150	200	~200
τ_{off} nBgTx (min)	<0.1	~120	<0.1	ND	<0.1	ND
αBgTx block	No	No	No	No	No	Yes

All data are from the heterologous expression of nAChRs in Xenopus oocytes. The nAChR subunit sequences were obtained from rat, unless otherwise noted. Reviews: Deneris et al., 1991; Chiappinelli, 1991; Bertrand et al., 1993a; McGehee and Role, 1995. References: Ballivet et al., 1988; Papke et al., 1989; Luetje et al., 1990; Cooper et al., 1991; Leutje and Patrick, 1991; Papke and Heinemann, 1991; Revah et al., 1991; Charnet et al., 1992; Bertrand et al., 1993b; Lester and Dani, 1993; Leutje et al., 1993; Gerzanich et al., 1994; Covernton et al., 1994; Connolly et al., 1995; Cohen et al., 1995; Ramirez-Latorre et al., 1996.

[a] Data from studies of chicken subunit cRNAs.

functional nAChRs. Although this approach provides the opportunity to test subunit function, the difficulties in proving specificity are considerable.

The most abundant nAChR species, which accounts for most of the high affinity nicotine binding in rat and chick CNS, is made up of the α4 and β2 gene products (Schoepfer et al., 1988; Whiting et al., 1987a, 1987b, 1991). In heterologous expression systems, the chick α4 and β2 gene products assemble in a two-to-three ratio to produce receptors (Anand et al., 1991; Cooper et al., 1991), preserving the structural motif of muscle nAChRs with two α and three non-α subunits (for review see Karlin and Akabas, 1996). Antisense oligonucleotide experiments confirm the participation of α4 in nAChRs expressed by specific populations of central, but not peripheral, neurons in chick (Listerud et al., 1991; Brussaard et al., 1994). Another major nAChR species, present in both the CNS and PNS, includes α7 subunits. The α7-containing nAChRs account for most of the high affinity α-bungarotoxin (αBgTx) binding, display a high relative permeability to Ca^{2+}, and desensitize rapidly (Couturier et al., 1990; Schoepfer et al., 1990; Bertrand et al., 1993b; Seguela et al., 1993; Vernallis et al., 1993; Zhang et al., 1994). Still a third major nAChR species, expressed primarily in autonomic neurons, is the more complex subtype composed of α3 subunits together with α5, β4, and sometimes β2 subunits (Listerud et al., 1991; Vernallis et al., 1993; Conroy and Berg, 1995; Ramirez-Latorre et al., 1996). Although other heteromeric and homomeric species exist in brain, they appear less abundant overall (for reviews see Sargent 1993; McGehee and Role, 1995). Individual nAChR gene products can assemble with different partners depending on the combinations available (Listerud et al., 1991; Conroy et al., 1992; Ramirez-Latorre et al., 1996). In view of these findings and the common observation that individual neurons express more than one nAChR channel subtype (e.g., Margiotta and Gurantz, 1989; Moss et al., 1989; Moss and Role, 1993; Alkondon and Albuquerque, 1993; Brussaard et al., 1994; Connolly et al., 1995), one clearly can not predict nAChR compositions based solely on the set of genes expressed by the neuron.

Functional Contributions of nAChRS in the CNS

Role of nAChRs in Early Developmental Events

The early presence of machinery both for synthesizing ACh and responding to it during early embryogenesis suggests important roles for nicotinic signaling early on in neural development. Choline acetyltransferase, the synthetic enzyme for ACh, has been found as early as neural plate stages in presumptive crest (Smith et al., 1979). Specific neuronal nAChR subunit transcripts are detected as early as embryonic day 2 throughout the mouse CNS (Zoli et al., 1995), and both subunit message and functional nAChRs are expressed in premigratory crest cells in vitro (Howard et al., 1995) and in autonomic and sensory neurons early in development (Corriveau and Berg, 1993; Arenella et al., 1993; Devay et al., 1994). The neuronal forms of nAChR subunits genes are also expressed in presumptive myoblasts and related cell types at early stages (Corriveau et al., 1995). The significance of nAChR expression prior to neuronal differentiation remains to be determined, but the high relative Ca^{2+} permeability of the subtypes expressed supports a role for nAChRs in regulating early gene expression. This has been suggested by the work of Greenberg and colleagues, who demonstrated regulation of c-fos transcript levels in a Ca^{2+}-dependent manner following nAChR activation in PC12 cells (Greenberg et al., 1986), and by recent studies implicating α7-type nAChRs in cell proliferation (Quik et al., 1994). Nicotinic AChR-mediated Ca^{2+} influx can activate second messenger cascades as evidenced by the release of arachidonic acid caused by stimulation of α7-containing nAChRs (Vijayaraghavan et al., 1995) and the activation of Ca^{2+}/calmodulin-dependent protein kinase in PC12 (MacNicol and Schulman, 1992). Recently, neuronal nAChRs have also been implicated in the regulation of motoneuron survival (Hory-Lee and Frank, 1995).

Neuronal nAChRs may also contribute to neuronal pathfinding and target selection. Application of an ACh gradient to growing neurites from Xenopus spinal neurons in cell culture can induce turning of the neurites up the gradient; the effect is blocked by nicotinic antagonists (Zheng et al., 1994). Pulses of ACh or nicotine

applied to growing neurites of chick ciliary ganglion neurons in culture can restrict growth and induce retraction; the effect is Ca^{2+}-dependent and blocked by αBgTx (Pugh and Berg, 1994). Cholinergic neurites release ACh spontaneously (Hume et al., 1983; Young and Poo, 1983). This could provide a feedback mechanism helping neurites assess location. If contact with a presumptive postsynaptic cell increases the rate of ACh release (Xie and Poo, 1986), the increased level of transmitter could activate growth cone nAChRs, altering the level of intracellular Ca^{2+} and thereby halting growth cone motility and stabilizing the location. In some cases, α7-containing nAChRs appear near dendrites (Jacob and Berg, 1983; Ullian and Sargent, 1995; Wilson Horch and Sargent, 1995) that subsequently undergo dramatic resorption into the cell body (Landmesser and Pilar, 1978); possibly, release of ACh from developing presynaptic terminals stimulates the receptors and helps drive the resorption.

Role of Postsynaptic nAChRs in CNS Transmission

Nicotinic transmission mediated by postsynaptic nAChRs is well established in autonomic ganglia as well as within the spinal cord at the Renshaw cell–motoneuron synapse and at efferent synapses on cochlear hair cells (e.g., Langley and Anderson, 1892; Belcher and Ryall, 1977; Elgoyhen et al., 1994; see Figures 1c and 1d). In contrast, it has been considerably more difficult to demonstrate sites in the brain where released ACh evokes postsynaptic nicotinic responses. Many neurons within the brain can generate fast inward currents in response to exogenously applied ACh: more than 50 such examples were noted at the time of Clarke's detailed review of the subject (Clarke, 1993) and still more have appeared since (e.g., Alkondon and Albuquerque, 1995; Connolly et al., 1995; Ishihara et al., 1995; Castro and Albuquerque, 1995). Indeed, several sites within the brain seemed to be prime candidates for nicotinic synaptic transmission based on the presence of cholinergic projections in the region of nAChR expressing neurons, but physiological study has largely revealed that synaptic transmission is not mediated by nAChRs even at these prime candidate sites for nicotinic transmission in the brain (e.g., Brown et al., 1983; Edwards et al., 1992).

Notable exceptions include the cholinergic projections of the zona intermedialis reticularis to a subset of neurons within the nucleus ambiguus where nicotinic transmission is reasonably well documented (Zhang et al., 1993). In addition, stimulation of central cholinergic pathways may enhance excitability of the dorsal motor nucleus of the vagus and the medial vestibular nucleus via direct nicotinic synapses (Ito et al., 1989; Phelan and Gallagher, 1992). The nicotinic activation of dopamine release in striatum may also be due, in part, to direct synaptic activation of nicotinic receptors on neurons of the substantia nigra (SNC), since focal stimulation of cholinergic inputs to the SNC (from the pedunculotegmental nucleus) evokes fast synaptic responses that are blocked by mecamylamine administered intravenously (Clarke et al., 1987; Futami et al., 1995).

The documentation of relatively few direct nicotinic synapses in the brain may be due to the inherent difficulties in studying central cholinergic synapses in situ. Cholinergic projections are diffuse, and the nAChR-containing targets are scattered through complex cortical, limbic, and central autonomic structures (for review see Woolf, 1991). In addition, nicotinic transmission in the CNS, like that of norepinephrine, may be dependent on coordinate activation of multiple afferent projections. Finally, the modulation of nAChR opening probability by external Ca^{2+}, recently elucidated by Dani and colleagues, is sufficent to alter the amplitude of cholinergic synaptic currents (Vernino et al., 1992; Amador and Dani, 1995). Thus, the millimolar decreases in Ca^{2+} produced by high levels of synaptic activity (e.g., Heinemann et al., 1990b; Livsey et al., 1990) may obscure the contribution of postsynaptic nAChRs to synaptic transmission in the brain.

Alternatively, it is possible that the difficulty in detecting fast excitatory nicotinic synapses in the brain reflects the participation of CNS nAChRs in functions other than the mediation of synaptic transmission per se. Important modulatory functions of nAChRs are indicated by studies demonstrating that the Ca^{2+} entry through these receptors is sufficient to activate Ca^{2+}-dependent chloride and potassium channels (Tokimasa and North, 1984; Fuchs and Murrow, 1992; Galzi et al., 1992; Vernino et al., 1992; Seguela et al., 1993) and can influence responses mediated by other ligand-gated ion channels such as γ-aminobutyric acid type A ($GABA_A$) receptors (Mulle et al., 1992a, 1992b).

Role of Presynaptic nAChRs in the Modulation of CNS Transmission

Nicotinic AChRs at presynaptic sites can modulate synaptic transmission by regulating the extent of transmitter release (Figures 1a and 1b). The targeting of nAChRs to synaptic terminals was implicated early on in a variety of biochemical and anatomical studies of specific CNS projections. Autoradiographic studies of both αBgTx- and nicotine-binding sites in the terminal fields of the medial habenula combined with lesioning of the fasiculus retroflexus suggested that as much as 50% of the αBgTx sites in the target interpeduncular nucleus might be presynaptic in origin (Clarke et al., 1986). The presence of high affinity nicotine sites on terminals of dopaminergic projections to striatum and accumbens (Clarke and Pert, 1985), as well as the role of these nAChRs in regulating release, is further implicated in studies of synaptosome preparations by Wonnacott, Collins, Clarke, and their colleagues (Rapier et al., 1988, 1990; Grady et al., 1992; Marks et al., 1993; El-Bizri and Clarke, 1994). Finally, the demonstration of axonal transport of high affinity nicotine-binding sites in CNS projections to tectum confirms the underlying mechanism for targeting of nAChRs to terminal fields (Henley et al., 1986). These and other studies of synaptosomal and brain slice preparations are consistent with presynaptic nAChRs enhancing the release of norepinephrine, dopamine, GABA, serotonin, and ACh (summarized in Table 2; Lapchak et al., 1989; King, 1990; Lena et al., 1993; Vidal and Changeux, 1993; Summers and Giacobini, 1995). Synaptosome studies, however, do not support a role of presynaptic nAChRs in regulating glutamate release, though it is not clear whether the assays of glutamate release employed would have been sufficient to resolve small changes (Wonnacott et al., 1995; S. Wonnacott,

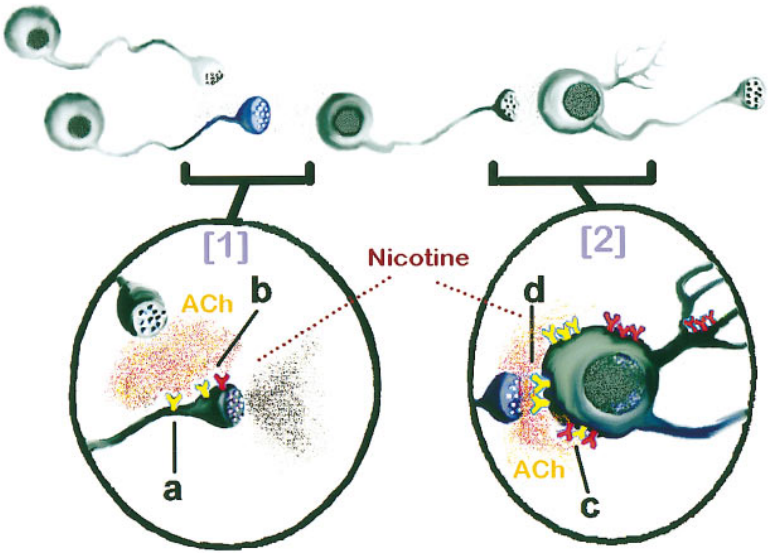


Figure 1. Model Illustrating Possible Locations and Corresponding Functions of Some nAChR Subtypes on Neurons in the CNS

Four neurons are shown. The first (left most) releases ACh onto the presynaptic terminal of a second that releases neurotransmitter (e.g., GABA, glutamate, ACh, dopamine, norepinephrine, or serotonin) at a classical synapse on the soma of a third; it, in turn, releases ACh at a somatic synapse on the fourth. Evidence from anatomical, biochemical, and physiological studies suggest both pre- and postsynaptic roles for heteromeric nAChRs containing α- and β-type subunits combined (yellow symbols) and for nAChRs containing α7 subunits (red symbols).

(Inset 1) Presynaptic nAChRs. Heteromeric α/β-containing receptors appear to be present within the "preterminal domain" (a), whereas both heteromeric and α7-containing receptors are likely to serve as presynaptic nAChRs on synaptic boutons (b).

(Inset 2) Postsynaptic nAChRs. On the postsynaptic neuron, α7-containing nAChRs are found clustered in perisynaptic locations as are heteromeric α/β-containing receptors (c), whereas only the latter appear to be concentrated in the immediate subsynaptic membrane (d) (see text and Ullian and Sargent, 1995; Wilson Horch and Sargent, 1995; Clarke 1993; and Vizi et al., 1995).

personal communication). In contrast, studies using extracellular stimulation and recording in cortex (Vidal and Changeux, 1989) or in the terminal fields of the fasiculus retroflexus (Brown et al., 1984) suggested that nicotine might enhance synaptic activity mediated by excitatory amino acid transmitters.

More comprehensive physiological studies have now extended previous work and further support the hypothesis that a significant role of CNS nAChRs may be to modulate, rather than to mediate, synaptic transmission (Lapchak et al., 1989; King, 1990; Lena et al., 1993; McMahon et al., 1994a, 1994b; Vizi et al., 1995; McGehee et al., 1995). Intracellular recording indicates that presynaptic nAChRs can modulate evoked (i.e., tetrodotoxin [TTX] sensitive) and spontaneous transmission at specific synapses dependent upon the targeting of nAChRs to terminal or preterminal sites (e.g., Lena et al., 1993; Figures 1a and 1b). Studies of GABA-mediated synaptic currents arising from terminals attached to dispersed interpeduncular nucleus (IPN) neurons reveal nAChRs may be targeted to the preterminal axon region where nicotinic agonists alter GABA release by a TTX-sensitive mechanism (Lena et al., 1993; McMahon et al., 1994a). Similar TTX-sensitive effects of nicotine on GABAergic transmission are seen in chick lateral spiriform nucleus (McMahon et al., 1994a). The pharmacology of the preterminal nAChRs implicated in TTX-blockable modulation is similar to α/β heteromeric nAChRs as it is blocked by mecamylamine and hexamethonium but not by αBgTx (Figure 1a; Table 2; Lena et al., 1993; McMahon et al., 1994a).

Recent biophysical studies combined with Ca^{2+}-imaging techniques demonstrate that nanomolar concentrations of nicotine can activate nAChRs and potently facilitate spontaneous and evoked glutamatergic synaptic transmission in CNS cultures (McGehee et al., 1995; Radcliffe and Dani, 1995, Soc. Neurosci., abstract). The nicotine-induced enhancement of excitatory transmission is presynaptic in origin, since the frequency but not the amplitude of spontaneous miniature synaptic currents is altered. Most striking is the unique pharmacology of nAChRs that enhance fast, excitatory transmission at these CNS synapses: the presynaptic nAChR complexes involved are blocked by αBgTx, and deletion experiments with antisense oligonucleotides indicate the nAChRs contain α7 subunits. The αBgTx-sensitive nAChRs that augment CNS glutamate and ACh release appear to be located on synaptic terminals per se, as exposure of presynaptic neurites to nicotine increases Ca^{2+} influx in the presence or absence of TTX or postsynaptic neurons. Thus, nicotinic modulation of presynaptic activity is independent of postsynaptically derived "retrograde messengers" and may induce sufficient Ca^{2+} influx to enhance vesicular release directly (McGehee et al., 1995). There is as yet, however, no direct evidence that the endogenous nicotinic agonist ACh is released at presumptive sites of presynaptic nAChR modulation. Although cholinergic projections are known to be present in many of the same terminal fields thought to contain presynaptic nAChRs (Table 2), it is clearly essential to demonstrate that stimulation of the cholinergic projection exerts the proposed modulation.

Future Directions

In view of the evidence accumulating that nAChRs can regulate transmitter release at many kinds of CNS synapses (dopaminergic, serotonergic, GABAergic, adrenergic, cholinergic, glutamatergic), it is attractive to consider that an important role of nAChRs in the brain may be to modify, rather than mediate, synaptic transmission. The proposed modulatory role for CNS nAChRs is not inconsistent with the relatively small number of nAChRs in the CNS. A judicious positioning of the receptors could enable them to participate in a broad range of behavioral and cognitive effects via modulation even

Table 2. Proposed Locations and Physiological Effects of Presynaptic nAChRs Relative to Projections of Central Cholinergic Nuclei

Location of Presynaptic nAChRs	Source of Cholinergic Input to nAChR Positive Terminal Fields	Effect of Activation of Presynaptic nAChRs	Presynaptic nAChR Subtype	Evidence
Glutamatergic terminals within the IPN	Medial hapenula N., diagonal band, laterodorsal tegmental N.	increase spontaneous and evoked glutamate release/ transmission	αBgTx sites, includes α7	Intracellular recording, direct stimulation of input, exogenous nicotinic agonist; blocked by αBgTx
GABAergic terminals within the IPN		Increase spontaneous GABA release	High affinity nicotine sites (α4β2??)	Intracellular recording, exogenous nicotinic agonist, not blocked by αBgTx
Terminals in prefrontal cortex	Nucleus basalis substantia innominata, N. ansa leticularis	Increase spike frequency [Increase EAA release]	High affinity nicotine sites (α4β2??)	Extracellular stimulation and recording field potentials, exogenous nicotinic agonist
Terminals in primary visual cortex		Increase spontaneous and evoked electrical activity in visual cortex [Increase EAA release]	High affinity nicotine sites (α4β2??)	Extracellular recording in visual cortex, light stimulus, exogenous nicotinic agonist
GABAergic terminals within the lateral spiriform N.	Local circuits?	Increase spontaneous GABA release	High affinity nicotine sites (α4β2??)	Intracellular recording exogenous nictotinic agonist
Dopaminergic terminals within the striatum and accumbens	Pontomesencephalic tegmental N.	Increase DA release	High affinity nicotine sites (α3β4??)	[^{3}H]DA release from synaptosomes, not blocked by αBgTx
Cholinergic terminals within the cerebellum	Medullary tegmental N.	Increase ACh release	High affinity nicotine sites (α4β2??)	ACh ''overflow'' from cerebellar slices
Noradrenergic and glutamatergic terminals within the hippocampus	Medial septal N., vertical diagonal band	Increase NE and glutamate release/transmission	Both high affinity nicotine and αBgTx sites	[^{3}H]NE release from synaptosomes, intracellular recording, exogenous nicotinic agonist, blocked by αBgTx
Cholinergic terminals within sympathetic ganglia	Visceral motoneurons (spinal cord)	Increase spontaneous and evoked ACh release/cholinergic transmission	Blocked by αBgTx, includes α7	Intracellular recording, direct stimulation of input, exogenous nicotinic agonist, blocked by aBgTx

Abbreviations: DA, dopamine; EAA, excitatory amino acid; N., nucleus; NE, norepinephrine. Examples of CNS projections and terminal fields where presynaptic nAChRs have been implicated in the regulation of the release of GABA, glutamate, NE, DA, and Ach. Note that this list is not comprehensive and that assignment of receptor sybtype is largely speculative (but see Grady et al., 1992). Reviews: Woolf, 1991; Clarke, 1993; Vizi et al., 1995; Wonnacott et al., 1995; Clarke et al., 1995. References: Langley and Anderson, 1892; Brown et al., 1984; Clarke and Pert, 1985; Clarke et al., 1986, 1987; Parkinson et al., 1988; Vidal and Changeaux, 1989; 1993; Rapier et al., 1988, 1990; King, 1990; Mulle et al., 1991; Schultz et al., 1991; Lena et al., 1993; Marks et al., 1993; Del-Toro et al., 1994; McMahon et al., 1994a, 1994b; McGehee et al., 1995; Radcliffe and Dani, 1995, Soc. Neurosci., abstract.

though the total number of nAChRs is several orders of magnitude below that of the main workhorses of the CNS, the glutamate and $GABA_A$ receptors. Targeting of nAChRs to axon/preterminal regions or synaptic boutons could allow these "high gain" structures to amplify greatly the actions of nicotinic agonists (Figure 1). Moreover, selective positioning of individual nAChR subtypes on such structures could generate different effects (e.g., Ca^{2+} influx to enhance transmitter release versus membrane depolarization to activate voltage-gated channels), depending on the receptor subtype.

Selective targeting of nAChRs within soma–dendritic domains may also be a critical determinant of receptor function (Figure 1). In these cases, close apposition to presynaptic transmitter release sites may utilize the cation-conducting properties of the receptors in the "classical" way to mediate fast, excitatory synaptic transmission. Segregation of highly Ca^{2+}-permeable nAChR subtypes, such as the α7-containing receptors, from one another in synaptic, perisynaptic, and extrasynaptic domains may allow them to direct different sets of cellular events using the same second messenger: Ca^{2+}. Further segregation of distinct nAChR channel subtypes to discrete high density receptor patches may encode more fine-tuned differences in the synaptic currents generated at these sites. A fundamental hypothesis underlying these views, and one that requires direct testing, is that individual nAChR subtypes can be selectively targeted to specific locations on the neuron.

Clearly, the role of CNS nAChRs are only beginning to be elucidated. Their importance is underscored by the effects of nicotinic agonists and antagonists on behavioral and cognitive functions. Nicotine enhances attention and arousal, diminishes anxiety, produces mild analgesia, and can even improve acquisition and retention of short term memories (for reviews see Clarke et al.,

1995). Although the impact of nicotine on performance in some test paradigms is controversial, the efficacy of nicotine in inducing dependence is not. Furthermore, recent studies of patients with cognitive or behavioral disorders such as Alzheimer's disease, Tourette syndrome, Parkinson's disease, and schizophrenia demonstrate significant alterations in the expression of CNS nAChRs and in some cases have shown therapeutic effects of nicotine administration (for reviews see London et al., 1989; Clarke et al., 1995; Schröder et al., 1995; James and Nordberg, 1995). The application of electrophysiology, Ca^{2+} imaging, and gene knockout technologies in the context of cognitive and behavioral testing (Picciotto et al., 1995) has begun a new era in probing the functions of CNS nAChRs.

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